

WHAT IS CLAIMED IS:

- 1                   1.     A method of creating a library of DNA sequences, said method  
2     comprising:
  - 3                   a)     providing a DNA sequence that encodes a protein of interest;
  - 4                   b)     providing a probability matrix for the protein;
  - 5                   c)     providing a constraint vector for the protein;
  - 6                   d)     applying the constraint vector to the probability matrix to produce a  
7     substitution scheme recommending substitutions at at least two residues in the protein;  
8     and
  - 9                   e)     creating a library of DNA sequences incorporating changes in the  
10    DNA sequence that produce the recommended substitutions.
- 1                   2.     The method of claim 1, wherein said protein is selected from the  
2     group consisting of an esterase, dehydrogenase and hydrolase.
- 1                   3.     The method of claim 2, wherein said protein is selected from the  
2     group consisting of a protease, cellulase, lipase, hemicellulase, laccase, and amylase.
- 1                   4.     The method of claim 1, wherein said protein is selected from the  
2     group consisting of a transcription factor, growth factor, antibody, interleukin, antigen,  
3     and receptor.
- 1                   5.     The method of claim 1, wherein the probability matrix is based on  
2     structural characteristics selected from the group consisting of conservative residues,  
3     sequence alignments, three dimensional structure, residue environment, solvent  
4     accessibility, residue chemistry, propensity for a particular secondary structure, and  
5     combinations thereof.
- 1                   6.     The method of claim 1, wherein the constraint vector is based on  
2     structural characteristics known to affect protein function selected from the group  
3     consisting of proximity to the site of functionality, distance of  $\alpha$  or  $\beta$  carbons, contact  
4     with residues of interest, and contact with residues that contact the residue of interest.

1                    7.        The library of claim 1, wherein said library is a phage library.

1                    8.        A method for screening a library for a protein with an increase in a  
2 property of interest, comprising:

3                    a)        providing a probability matrix for a protein of interest;  
4                    b)        providing a constraint vector for the protein;  
5                    c)        applying the constraint vector to the probability matrix to produce a  
6 substitution scheme recommending substitutions at at least two residues in the protein;  
7 and

8                    d)        creating a library of DNA sequences incorporating changes in the  
9 DNA sequence that produce the recommended substitutions; and

10                   e)        screening the library for a protein with an increase in the property  
11 of interest.

1                    9.        The method of claim 8, further comprising identifying a protein  
2 having an increase in the property of interest.

1                    10.       A protein produced by the method of claim 9.

1                    11.       A system for creating libraries of nucleic acid sequences that  
2 encode variants of a protein, said system comprising:

3                    a)        an initial nucleic acid sequence that encodes a desired protein;  
4                    b)        a probability matrix; and  
5                    c)        a constraint vector.

1                    12.       A method for improving a desired parameter of a protein of  
2 interest, comprising:

3                    a)        providing a probability matrix for the desired protein;  
4                    b)        providing a constraint vector for the desired protein;  
5                    c)        applying the constraint vector to the probability matrix to produce a  
6 substitution scheme recommending substitutions at at least two residues in the protein;  
7 and

8                    d)        creating a library of DNA sequences incorporating changes in the

- 9 DNA sequence that produce the recommended substitutions; and
- 10 e) measuring the parameter of interest for at least two members of
- 11 said library;
- 12 f) determining the sequence for at least two members of said library;
- 13 and
- 14 g) using sequence comparison and correlation analysis to determine
- 15 the contribution of mutations or combination of mutations on the parameter measured in
- 16 step e).

1 13. The method of claim 12, wherein the contribution of mutations

2 determined in step g) is used to generate a second library.

1 14. The method of claim 1, wherein a library comprising at least 25

2 unique DNA sequences is produced.

1 15. The method of claim 14, wherein a library comprising at least 100

2 unique DNA sequences is produced.

1 16. The method of claim 15, wherein a library comprising at least 250

2 unique DNA sequences is produced.

1 17. The method of claim 16, wherein a library comprising at least 1000

2 unique DNA sequences is produced.

1 18. The method of claim 17, wherein a library comprising at least 2500

2 unique DNA sequences is produced.

1 19. The method of claim 18, wherein a library comprising at least

2 10,000 unique DNA sequences is produced.

1 20. The method of claim 1, wherein a library of less than  $10^9$  unique

2 DNA sequences is produced.

1 21. The method of claim 20, wherein a library of less than  $10^6$  unique

2 DNA sequences is produced.

1 22. The method of claim 21, wherein a library of less than  $10^5$  unique

2 DNA sequences is produced.

1 23. The method of claim 1, wherein the probability matrix is an